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THE UNITED STATES PATENT AND TRADEMARK OFFICE
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

Appeal No:

In re the Application of: **KATO et al.**

Group Art Unit:**1643**

Serial No.: **09/340,196**

Examiner: **HOLLERAN, Anne L.**

Filed: **June 28, 1999**

P.T.O. Confirmation Nos.: **3596**

For: **METHOD FOR MEASURING THYROGLOBULIN**

BRIEF ON APPEAL

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

April 30, 2007

Sir:

This is an appeal from the Office Action dated December 1, 2006 in which claims 59, 68-75, 77 and 78 were finally rejected.

A Notice of Appeal was timely filed on March 1, 2007.

TABLE OF CONTENTS

I. REAL PARTY IN INTEREST

The real party in interest is the assignee of the subject application, which is:

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Chuo-ku, Osaka 540-8605 JAPAN

II. RELATED APPEALS AND INTERFERENCES

Appellants know of no other appeals or interference proceedings related to the present appeal.

III. STATUS OF CLAIMS

Claims 59, 68-75, 77 and 78 on appeal have been finally rejected and are the subject of this appeal.

IV. STATUS OF AMENDMENTS

All previous amendments have been entered.

V. SUMMARY OF THE CLAIMED SUBJECT MATTER

The invention as now claimed, is a method for determining between a malignant thyroid tumor and a benign thyroid tumor having the following general steps:

(1) measuring an amount of one of two types of thyroglobulin in a fluid sample originating from a living body, the steps comprising:

(a) adding to the sample a specific lectin capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin, to form a conjugate of the specific lectin with the first type of thyroglobulin;

(b) separating said conjugate from the non-conjugated second type of thyroglobulin;

(c) measuring said conjugate content by adding a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin, for determining the amount of the first type of thyroglobulin; or

(d) measuring an amount of the non-conjugated second type of thyroglobulin by adding a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin,

(2) determining whether the thyroid tumor is malignant or benign by comparing a calculated ratio of the amount measured in (c) or (d) to an amount of total thyroglobulin in the sample with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) and (ii),

(i) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid, or

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, and

the sample is determined to be benign in the following case (iii):

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the malignant thyroid.

VI. OUTSTANDING ISSUES AND GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL

1. Whether or not Claims 59, 68, 69, 74 and 77 on appeal are unpatentableunder 35 USC 103(a) as being obvious over either Nakamura (US Patent 5,571,729; issued 11/5/1996) or Satomura (US Patent 5,780,247, issued 7/14/1998; effective filing 1/5/1991) in view of either Yamamoto (of record), Tarutani (of record), or Survilo (Surivilo, L.I. et al., Vestsi Akademii Navuk Belarusi, Seryya Khimichnykh Navuk, 4:103-107, 1997; abstract only).

2. Whether or not Claims 70, 71, 77 and 78 on appeal are unpatentable under 35 USC 103(a) as being obvious over Katoh (US Patent 5,591,589; issued 1/7/1997) in view of either Yamamoto (of record), Tarutani (of record), or Survilo (Surivilo, L.I. et al., Vestsi Akademii Navuk Belarusi, Seryya Khimichnykh Navuk, 4:103-107, 1997; abstract only).

3. Whether or not Claims 73 and 77 on appeal are unpatentable under 35 USC 103(a) as being obvious over Canfield (WO/87/00289;) in view of Yamamoto (of record).

4. Whether or not Claims 72, 75 and 77 on appeal are unpatentable under 35 USC 103(a) as being obvious over Katoh (supra) in view of Canfield(WO/87/00289) and further in view of Yamamoto(supra).

5. Whether or not Claims 59, 68, 69, and 74 on appeal are unpatentable under the judicially created doctrine of obviousness-type double patenting over claims 1, and 5-9 of US Patent No.5,780,247 in view of either Yamamoto (of record), Tarutani (of record) or Survilo (Surivilo, L.I. et al., Vestsi Akademii Navuk Belarusi, Seryya Khimichnykh Navuk, 4:103-107, 1997; abstract only).

6. Whether or not Claims 70, 71 and 78 on appeal are unpatentable under the judicially created doctrine of obviousness-type double patenting over claims 1 and 3 of US Patent No.5,591,589 in view of either Yamamoto (of record), Tarutani (of record) or Survilo(Surivilo, L.I. et al., Vestsi Akademii Navuk Belarusi, Seryya Khimichnykh Navuk, 4:103-107, 1997; abstract only).

VII. ARGUMENT

One of the primary differences between the invention as now claimed and the prior art is that the determination of malignancy of the thyroid tumor is not determining whether the thyroid is "**normal or malignant**", but determining whether the thyroid tumor is "**benign or malignant**."

For instance, whether a thyroid sample is normal or tumor (whether a thyroid sample is normal or abnormal) can be determined only by common practices such as X-ray examination or naked eye examination. However, it is difficult to determine **whether the thyroid tumor is benign or malignant** only by such common practices.

Therefore, the purpose of the claimed invention is to determine whether the thyroid tumor is "**benign or malignant**" (that is, **malignancy** of the thyroid tumor). So, since the present invention can determine whether the tumor thyroid is benign or malignant, it is a significant clinical advancement.

A. Claims 59, 68, 69, 74 and 77 on appeal are patentable over either Nakamura (US Patent 5,571,729; issued 11/5/1996) or Satomura (US Patent 5,780,247, issued 7/14/1998; effective filing 1/5/1991) in view of either Yamamoto (of record), Tarutani (of record), or Survilo (Surivilo, L.I. et al., Vestsi Akademii Navuk Belarusi, Seryya Khimichnykh Navuk, 4:103-107, 1997; abstract only).

Nakamura, Satomura, Yamamoto, Survilo do not teach a method for determining

malignancy of thyroid tumor by using a **ratio**. Moreover, Tarutani does not disclose the ratio recited in the present claims, that is, of [Con A-gel unbound Tg or Con A-gel bound Tg] / [the total Tg].

The Examiner asserts that Yamamoto teaches that thyroglobulin isolated from malignant thyroid tumor tissue has a different DEAE-cellulose ion exchange elution pattern from thyroglobulin isolated from benign and from normal thyroids (April 8, 2004 Office action p.9, lines 19-21). However, Yamamoto is silent on any **ratio**.

Yamamoto does not show the measurement of an amount of thyroglobulin. On p.135, Fig. 1, of Yamamoto, human thyroglobulin was purified, **digested by the action of exhaustive pronase**, subjected to DEAE-cellulose ion exchange chromatography (Yamamoto, p.133, column 2, MATERIALS AND METHODS, *Isolation of oligosaccharid.es from human thyroglobulin,* p.134, column 1, *Fractionation of oligosaccharides).* On p.139, Fig. 5, of Yamamoto, purified thyroglobulin was **digested by the action of exhaustive pronase**, subjected to DEAE-cellulose ion exchange chromatography, eluted with a linear concentration gradient of NaCl, subjected to Con A-Sepharose chromatography and RCA-Sepharose chromatography (p.134, column 1, *Fractionation of oligosaccharides).* That is, Yamamoto compares the elution pattern of **digested product** of thyroglobulin from a malignant thyroid tissue with that of digested product of thyroglobulin from normal thyroid tissue.

That is, Yamamoto **does not** measure the amount of thyroglobulin, nor compare an amount of thyroglobulin itself from a malignant thyroid tissue with that from a normal thyroid tissue. Appellant therefore disagrees with the Examiner (April 8, 2004 Office action page 10, lines 12-15)

that Yamamoto provides teachings that allow one to predict that lectin affinity may be used as the basis for an assay to differentiate thyroglobulin secreted from a thyroid tumor from thyroglobulin secreted from a nón-cancerous thyroid.

The Examiner asserts that Tarutani teaches that the percent of total thyroglobulin that binds to Con-A is different for trabecular carcinoma compared to either follicular adenoma (a benign condition) or normal thyroid tissue (see page 855, Table II) (April 8, 2004 Office action p. 11, lines 1-3).

Although Tarutani shows the ratio of [Con A-gel unbound Tg or Con A-gel bound Tg] / [the total Tg] from malignant thyroid tissue and from a normal thyroid tissue and that from a benign thyroid tissue (Tarutani, p.855, TABLE II), used amounts as thyroglobulin are derived from water soluble protein but not thyroglobulin itself. Tarutani, p.852, left column under *"Thyroid Glands,"* states:

> "Thyroid tissues were sliced and soluble proteins were extracted in the buffer used for the con A-gel affinity chromatography. The supernatant, obtained after centrifugation, was used to prepare thyroglobulin."

And, under *"Isolation of thyroglobulin on a Concanavalin A-Sepharose column,"* states:

> "A solution of the thyroid extract was applied to the column, and the unadsorbed protein was removed by extensive washing with the buffer. Protein adsorbed on the column was eluted with MeG dissolved in the buffer."

In addition, Tarutani measures an amount of thyroglobulin as the amount of water soluble protein by absorbance of $E^{1\%,\,1\,cm}_{280nm}$ (Tarutani, p. 852, column 2, lines 1-2.)

Therefore, **Tarutani does not disclose the measurement of thyroglobulin using anti thyroglobulin antibody** and the ratio of [Con A-gel unbound Tg or Con A-gel bound Tg] / [the total Tg] as in the present claims.

The Examiner suggests that there is no difference in the present invention and the prior art, like Tarutani, because in Figs. 1 to 3 of the present specification, there is a malignant sample of which the ratio is overlapping with the ratio of benign sample. **However, this is a misunderstanding of the point of novelty of the claimed invention.** The claimed invention is that benign and malignant can be distinguished by using the ratio of a specific thyroglobulin using the present method as explained below.

In Figs.1 to 3, there are parts that overlap in normal or benign and malignant. However, other than at the end points, the ratios of malignant have not overlapped the ratios of benign. **That is, excluding the endpoints (end of the range), the ratio of malignant does not overlap with the ratio of benign in Figs.1 to 3.** Thus, it is possible to determine whether a sample is malignant but not benign. For instance, when the ratio of the sample is higher than the ratio of benign, the sample can positively be diagnosed malignant.

The percent amount that the ratio of malignant is overlapped with the ratio of normal or benign in each Fig.1 to 3 of the present specification was shown in the following table:

	Total Number	Number of data that overlapped with benign (%)	Number of data that overlapped with normal (%)
Fig.1	11	1 (9.1%)	1 (9.1%)
Fig.2	8	1 (12.5%)	3 (37.5%)

Fig.3	4	0 (0%)	1 (25%)

As is clear from the above table, the percentage of sample of which the ratio of malignant overlapped with the ratio of benign is less than 13%, based on the data presented in the specification. In other words, other samples of which the ratio does not overlapped with the benign can be determined as malignant but not benign.

In contrast, the ratio of benign is a ratio in the vicinity of the middle within the range of the ratio of malignant in Tarutani (Table II). **That is, there is no range where the sample can be determined as malignant but not benign, at all.**

Therefore, it is impossible to distinguish the malignant from benign by the method of Tarutani.

Additionally, in the Table II of Tarutani, ratio of Tg(%)(Unbound) in normal thyroid tissue of Group III (Patient NH) is 0 (Unbound). The ratio in papillary carcinoma (Patient RS, primary) of Group III is 0 (Unbound), too. This means that the ratio of normal is as same as that of papillary carcinoma. From this result of Tarutani, even though the ordinary skilled in the art will not be able to predict that the ratio of [an amount of first type or second type of thyroglobulin]/[total amount of thyroglobulin] is useful for determining the malignancy of thyroid disease (benign or malignant).

B. Claims 70, 71, 77 and 78 are patentable over Katoh (US Patent 5,591,589; issued 1/7/1997) in view of either Yamamoto (of record), Tarutani (of record), or Survilo (Surivilo,

L.I. et al., Vestsi Akademii Navuk Belarusi, Seryya Khimichnykh Navuk, 4:103-107, 1997; abstract only).

Appellant submits that Katoh, Yamamoto, Survilo do not teach the method for determining malignancy of thyroid tumor by using the recited **ratio**. In particular, as discussed above, Tarutani does not teach the use of the ratio [Con A-gel unbound Tg or Con A-gel bound Tg] / [the total Tg], as recited in the present claims, for determining malignancy of a thyroid tumor.

In response to the repeated rejection, the appellant has analyzed the data in the specification to make it comparable to that of the prior art, for example Tarutani, to clearly show the difference between the claimed invention and that in the prior art.

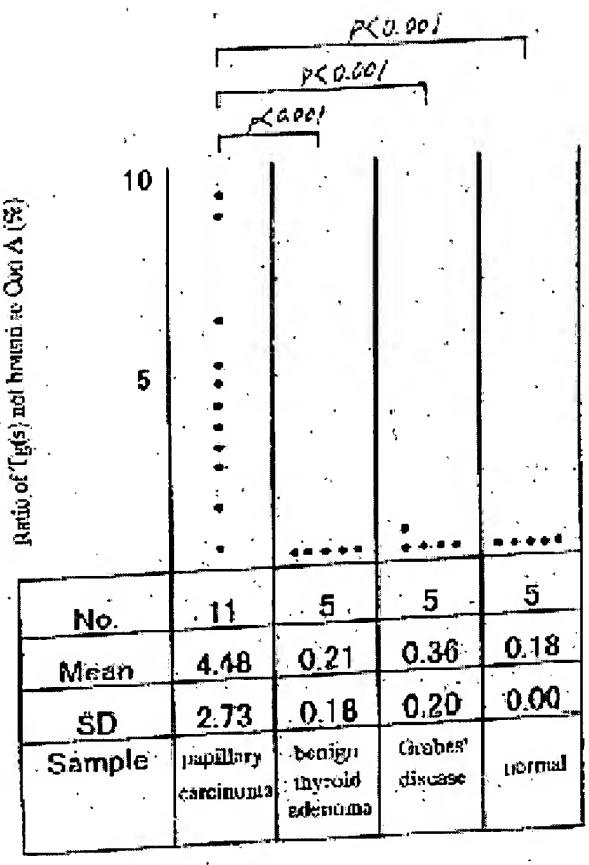
First, Figs. 1 to3 of the present specification have been prepared showing the results of statistical analysis (error analysis) of a two-sample t test (Welch's method). The results of statistical analysis are referred to by a reference (Arch Pathol Lab Med. 1998 Aug; 122(8): 715-720). A copy of the reference is enclosed. Note that the first author, Maruyama M, is one of the present inventors, and the publication date (August 1998) is later than the priority date of the present application (June 30, 1998). The left figure of Fig. 1 of the reference corresponds to Fig. 1 of the appellant's specification. The right figure of Fig. 1 of Maruyama corresponds to Fig. 2 of the present specification and Fig. 2 of Maruyama corresponds to the appellant's Fig. 3. The discussion of the Figures is on p.717, right column, line 1 to p.718, left column, line 20.

A two-sample t test (Welch's method) is used for analyzing the difference of means between two samples by using significance probability (p). The lower value of significance probability means the higher significant difference between two samples. Generally, significance probability between two

samples is lower than *5%* ($p<0.05$), it is determined that there is significant difference between two samples.

Fig.1: The ratio of Tg(s) not bound to Con A (%) was 4.48 ± 2.73% (mean ± SD; n=11) for papillary carcinoma, 0.21 + 0.18% (n=5) for benign thyroid adenoma, 0.36 ± 0.20% (n=5) for Graves' disease, and 0. ±0.00% (n=5) for normal tissues. The values for papillary carcinoma tissues were significantly higher than those for Graves' disease, benign thyroid adenoma, and normal tissues ($p<0.001$).

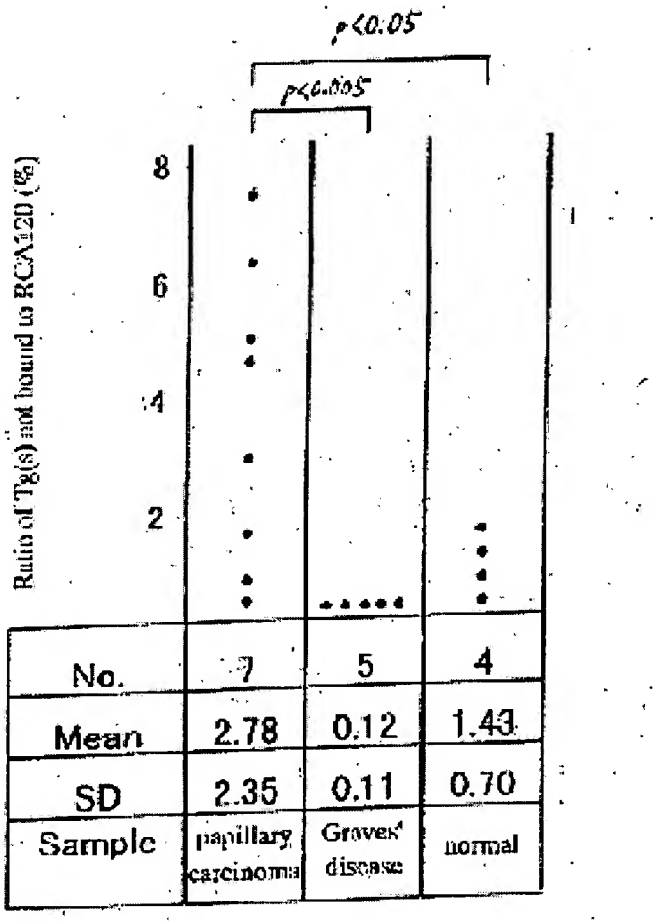
Fig. 1



No.	11	5	5	5
Mean	4.48	0.21	0.36	0.18
SD	2.73	0.18	0.20	0.00
Sample	papillary carcinoma	benign thyroid adenoma	Graves' disease	normal

Fig.2: The ratio of Tg(s) not bound to RCA120 (%) was 2.78 ± 2.35% (mean ±SD, n=7) for papillary carcinoma, 0.12 + 0.11% (n=5) for Graves' disease, and 1.43 ± 0.70% (n=4) for normal tissue. In the case of RCA-120, the differences between the ratio of Tg(s) not bound to RCA120 (%) for papillary carcinoma and Graves' disease ($p < 0.005$), as well as between papillary carcinoma and normal tissues ($p < 0.05$), were statistically significant.

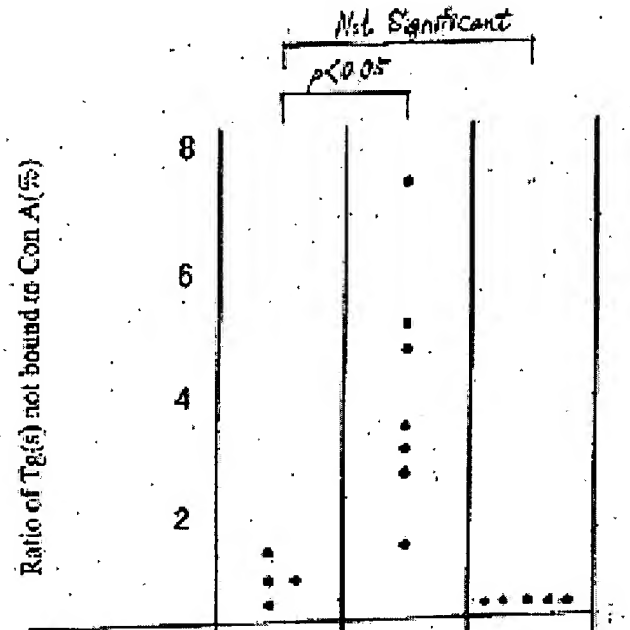
Fig. 2



No.	7	5	4
Mean	2.78	0.12	1.43
SD	2.35	0.11	0.70
Sample	papillary carcinoma	Graves' disease	normal

Fig.3: The ratio of Tg (s) not bound to Con A (%) was 0.83 ± 0.32% (n=4) for follicular carcinoma, 3.82 ± 2.12% (n=7) for follicular adenoma, and 0.18 ± 0.00% (n=5) for normal tissues. The values for the follicular adenoma were significantly higher than those for follicular carcinoma ($p<0.05$), however, there were no differences in unbound Tg(%) between follicular carcinoma and normal thyroid tissues.

Fig. 3

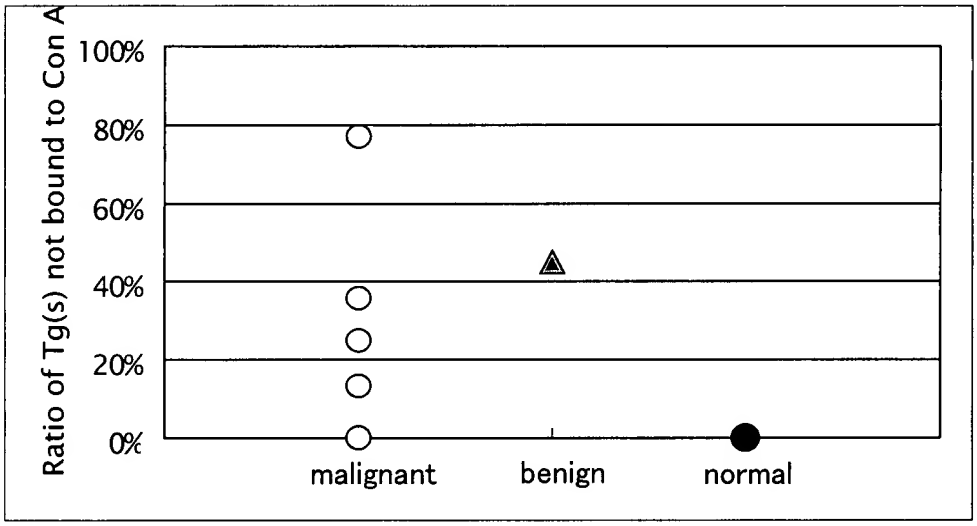


No.	4	7	5
Mean	0.83	3.82	0.18
SD	0.32	2.12	0.00
Sample	follicular carcinoma	follicular adenoma	normal

Thus, the analysis above proves that within a small margin or error, the appellant's claimed method is viable, whereas no such method is disclosed anywhere in the combination of prior art references. In fact, the data shown by Tarutani does not yield results according to the claimed method, as shown below.

Secondly, in order to compare the Tarutani Figure mentioned above with Fig.1 and Fig.3 of the present invention directly according to the Examiners' request, a Figure, in which the appellant has rewritten the data mentioned in Table II of Tarutani, is presented as follows: That is, No.1 of X-axis changed from 1 (normal) to malignant, No.3 of X-axis changed from 3 (malignant) to normal. Regimen of Y-axis was changed to the same with the figures of the present invention (ratio of Tg(s) not bound to Con A (%)).

(New Figure of Tarutani)



First, in Fig.1 of the present invention, the 1st column (the most left column) is related to papillary carcinoma (malignant), the 2nd and 3rd column are related to benign thyroid adenoma and

Graves' disease, respectively (benign), and the 4th column (the most right column) is related to normal. As is clear from Fig. 1, the malignant data are clearly higher than the benign data.

In Fig.3 of the present invention, the 1st column (the left column) is related to follicular carcinoma (malignant), the 2nd column (the middle column) is related to follicular adenoma (benign), and the 3rd column (the right column) is related to normal. As is clear from Fig. 3, the malignant data are clearly lower than the benign data.

In contrast, the benign data is in the middle of the range of the malignant data in the new figure of Tarutani, so that malignant cannot be distinguished from benign.

And therefore, from the disclosure of Tarutani, the skilled artisan will understand that it will be **impossible** to determine the thyroid tumor is benign or malignant (that is, the malignancy).

From the above results, it is clear that malignant can be distinguished from benign by the claimed invention, though the malignant cannot be distinguished from benign by the data of Tarutani.

The difference between the claimed invention and Tarutani can also be explained as a difference of measuring method of the thyroglobulin.

Tarutani measures an amount of water-soluble protein using absorbance of $E^{1\%,1cm}_{280nm}$, and the obtained amount is assumed to be the amount of Tg. The thus obtained soluble protein amount may contain Tg and other water-soluble proteins. That is, Tarutani does not quantify the Tg amount specifically. Therefore, the amount of water-soluble protein is not equal to the amount of Tg.

On the other hand, the claimed invention measures an amount of Tg

specifically by immunoassay using the anti-Tg antibodies. Therefore, the obtained amount is equal to the amount of Tg itself.

Therefore, based on an analysis of the appellant's data compared with that shown in the prior art as well as a comparison of the methods disclosed in the prior art, the appellant asserts that references in combination, Katoh, Yamamoto, Tarutani, or Survilo, cannot possibly disclose or make obvious the invention as now claimed.

C. Claims 73 and 77 are patentable over Canfield (WO/87/00289;) in view of Yamamoto (of record).

Claim 73 requires in step (d) calculating a ratio of the amount of the first type of thyroglobulin (or of the second type of thyroglobulin) to the amount of total thyroglobulin.

Appellant has discussed Yamamoto above, arguing that Yamamoto does not teach a method for determining malignancy using a **ratio**. Appellant likewise submits that Canfield does not teach a method for determining malignancy of thyroid tumor by using a **ratio**. Appellant therefore submits that no combination of Canfield and Yamamoto can provide the limitations of claims 73 and 77, and claims 73 and 77 are not obvious over the cited references, taken separately or in combination.

D. Claims 72, 75 and 77 are patentable over Katoh (supra) in view of Canfield (WO/87/00289) and further in view of Yamamoto (supra).

Appellant has discussed above that Canfield and Yamamoto do not teach a method for determining malignancy of thyroid tumor by using a **ratio** as in the present claims. Appellant similarly asserts that Katoh does not disclose a method using a **ratio** as in claims 72, 75 and 77. Claims 72, 75 and 77 are not obvious over the cited references, taken separately or in combination.

E. Claims 59, 68, 69, and 74 are patentable over claims 1, and 5-9 of US Patent No.5,780,247 in view of either Yamamoto (of record), Tarutani (of record) or Survilo (Surivilo, L.I. et al., Vestsi Akademii Navuk Belarusi, Seryya Khimichnykh Navuk, 4:103-107, 1997; abstract only).

The appellant acknowledges this double patenting rejection and asserts that a terminal disclaimer can be filed to overcome the rejection.

F. Claims 70, 71 and 78 are patentable over claims 1 and 3 of US Patent No.5,591,589 in view of either Yamamoto (of record), Tarutani (of record) or Survilo(Surivilo, L.I. et al., Vestsi Akademii Navuk Belarusi, Seryya Khimichnykh Navuk, 4:103-107, 1997; abstract only).

The appellant acknowledges this double patenting rejection and asserts that a terminal disclaimer can be filed to overcome the rejection.

G. CONCLUSION

The appellant respectfully requests that the final rejection be withdrawn and the claims be allowed and passed to issue, pending the filing of two Terminal Disclaimers to overcome the two double patenting rejections.

In the event this paper is not timely filed, appellant hereby petitions for an appropriate extension of time. The fee for any such extension may be charged to our Deposit Account No. 01-2340, along with any other additional fees which may be required with respect to this paper.

Respectfully submitted,

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Enclosures: Claims Appendix
Evidence Appendix
Related Proceedings Appendix

VIII. CLAIMS APPENDIX

Claims 59, 68-75, 77 and 78 are listed as still pending below.

Claims 1-58 (Canceled).

Claim 59 (Previously Presented): A method for determining between a malignant thyroid tumor and a benign thyroid tumor comprising:

(1) measuring an amount of one of two types of thyroglobulin in a fluid sample originating from a living body, the steps comprising:

(a) adding to the sample a specific lectin capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin, to form a conjugate of the specific lectin with the first type of thyroglobulin;

(b) separating said conjugate from the non-conjugated second type of thyroglobulin;

(c) measuring said conjugate content by adding a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin, for determining the amount of the first type of thyroglobulin; or

(d) measuring an amount of the non-conjugated second type of thyroglobulin by adding a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin,

(2) determining whether the thyroid tumor is malignant or benign by comparing a calculated ratio of the amount measured in (c) or (d) to an amount of total thyroglobulin in the sample with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) and (ii),

(i) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid, or

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, and

the sample is determined to be benign in the following case (iii):

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the malignant thyroid.

Claims 60 - 67 (Canceled).

Claim 68 (Previously Presented): A method for determining between a malignant thyroid tumor and a benign thyroid tumor comprising the steps of:

(a) adding to a fluid sample originating from a living body:

(i) a specific lectin capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin, and

(ii) a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin, to form a first conjugate which is a conjugate of the first anti-thyroglobulin antibody with the first type of thyroglobulin and with the

specific lectin, and a second conjugate which is a conjugate of the first anti-thyroglobulin antibody with the second type of thyroglobulin;

(b) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content; and

(c) measuring an amount of the second type of thyroglobulin on the basis of the second conjugate content;

(d) calculating a ratio of the amount of the first type of thyroglobulin measured in (b) to the amount of total thyroglobulin; or the amount of second type of thyroglobulin measured in (c) to the amount of total thyroglobulin; and

(e) determining whether a thyroid tumor is malignant or benign by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) and (ii),

(i) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid, or

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, and

the sample is determined to be benign in the following case (iii):

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the

malignant thyroid.

Claim 69 (Previously Presented): A method for determining between a malignant thyroid tumor and a benign thyroid tumor comprising the steps of:

(a) adding to a fluid sample originating from a living body, a specific lectin capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin,

to form a conjugate of the specific lectin with the first type of thyroglobulin;

(b) separating the conjugate from the second type of thyroglobulin; and

(c) measuring an amount of the first type of thyroglobulin on the basis of the conjugate content by adding a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin; and

(d) measuring an amount of the separated second type of thyroglobulin by adding a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin;

(e) calculating a ratio of the amount of the first type of thyroglobulin measured in (c) to the amount of total thyroglobulin; or the amount of second type of thyroglobulin measured in (d) to the amount of total thyroglobulin; and

(f)determining whether a thyroid tumor is malignant or benign by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) and (ii),

(i) when the calculated ratio is significantly higher than that of the reference fluid sample

of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid, or

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, and

the sample is determined to be benign in the following case (iii):

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the malignant thyroid.

Claim 70 (Previously Presented): A method for determining between a malignant thyroid tumor and a benign thyroid tumor comprising the steps of:

(a) adding to a fluid sample originating from a living body:

(i) a specific lectin capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin,

(ii) a first anti-thyroglobulin antibody capable of binding to both the first type of thyroglobulin and the second type of thyroglobulin, and

(iii) a second anti-thyroglobulin antibody capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin is already bound,

to form a first conjugate which is a conjugate of the first anti-thyroglobulin antibody with the first type of thyroglobulin and with the specific lectin, and a second conjugate which is a

conjugate of the first anti-thyroglobulin antibody with the second type of thyroglobulin and the second anti-thyroglobulin antibody;

(b) separating the first conjugate and the second conjugate; and

(c) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content; and

(d) measuring an amount of the second type of thyroglobulin on the basis of the second conjugate content;

(e) calculating a ratio of the amount of the first type of thyroglobulin measured in (c) to the amount of total thyroglobulin; or the amount of second type of thyroglobulin measured in (d) to the amount of total thyroglobulin; and

(f) determining whether a thyroid tumor is malignant or benign by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) and (ii),

(i) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid, or

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, and

the sample is determined to be benign in the following case (iii):

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample

of the normal thyroid and is significantly lower than that of the reference fluid sample of the malignant thyroid.

Claim 71 (Previously Presented): A method for determining between a malignant thyroid tumor and a benign thyroid tumor comprising the steps of:

(a) adding to a sample originating from a living body:

(i) a specific lectin capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin, and

(ii) an anti-thyroglobulin antibody-2 capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin is already bound,

to form a first conjugate which is a conjugate of the specific lectin with the first type of thyroglobulin, and a second conjugate which is a conjugate of the anti-thyroglobulin antibody-2 with the second type of thyroglobulin;

(b) separating the first conjugate and the second conjugate formed in the step (a);

(c) adding an anti-thyroglobulin antibody-1 capable of binding to both types of thyroglobulin to the second conjugate formed in the step (a), to form a third conjugate which is a conjugate of the anti-thyroglobulin antibody-2 with the second type of thyroglobulin and with the anti-thyroglobulin antibody-1;

(d) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content and

(e) measuring an amount of the second type of thyroglobulin on the basis of the third

conjugate content;

(f) calculating a ratio of the amount of the first type of thyroglobulin measured in (d) to the amount of total thyroglobulin; or the amount of second type of thyroglobulin measured in (e) to the amount of total thyroglobulin; and

(g) determining whether a thyroid tumor is malignant or benign by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) and (ii),

(i) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid, or

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, and

the sample is determined to be benign in the following case (iii):

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the malignant thyroid.

Claim 72 (Previously Presented): A method for determining between a malignant thyroid

tumor and a benign thyroid tumor comprising the steps of:

(a) adding to a sample originating from a living body:

(i) a specific lectin capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin, and (ii) an anti-thyroglobulin antibody-2 capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin is already bound,

to form a first conjugate which is a conjugate of the specific lectin with the first type of thyroglobulin, and a second conjugate which is a conjugate of the anti-thyroglobulin antibody-2 with the second type of thyroglobulin;

(b) separating the first conjugate and the second conjugate; and

(c) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content; and

(d) measuring an amount of the second type of thyroglobulin on the basis of the second conjugate content;

(e) calculating a ratio of the amount of the first type of thyroglobulin measured in (c) to the amount of total thyroglobulin; or the amount of second type of thyroglobulin measured in (d) to the amount of total thyroglobulin; and

(f) determining whether a thyroid tumor is malignant or benign by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample

originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) and (ii),

(i) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid, or

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, and

the sample is determined to be benign in the following case (iii):

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the malignant thyroid.

Claim 73 (Previously Presented): A method for determining between a malignant thyroid tumor and a benign thyroid tumor comprising the steps of:

(a) dividing a fluid sample originating from a living body into a first portion and a second portion;

(b)(i) adding to the first portion a specific lectin capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin,

to permit the precipitation of a conjugate of the first type of thyroglobulin with the specific

lectin;

(ii) separating the precipitated conjugate from the second type of thyroglobulin; and

(iii) measuring an amount of the second type of thyroglobulin of the separated part of first portion by adding a first anti-thyroglobulin antibody capable of binding to both types of the thyroglobulin; and

(c)(i) measuring an amount of the total thyroglobulin of the second portion; and

(ii) determining an amount of the first type of thyroglobulin from the difference between an amount of the total thyroglobulin and the amount of the second type of thyroglobulin obtained in step (b)(iii);

(d) calculating a ratio of the amount of the first type of thyroglobulin measured in (c)(ii) to the amount of total thyroglobulin; or the amount of second type of thyroglobulin measured in (b)(iii) to the amount of total thyroglobulin; and

(e)determining whether a thyroid tumor is malignant or benign by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) and (ii),

(i) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid, or

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample

of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, and

the sample is determined to be benign in the following case (iii):

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the malignant thyroid.

Claim 74 (Previously Presented): A method for determining between a malignant thyroid tumor and a benign thyroid tumor comprising the steps of:

(a) adding to a fluid sample originating from a living body, a specific lectin capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin; then

(b) adding to the sample a first antibody, capable of binding to both types of thyroglobulin, to form a first conjugate which is a conjugate of the first antibody with the first type of thyroglobulin and with the specific lectin, and a second conjugate which is a conjugate of the first antibody with the second type of thyroglobulin;

(c) separating the first conjugate and the second conjugate; and

(d) measuring an amount of the first type of thyroglobulin on the basis of the first conjugate content; and

(e) measuring an amount of the second type of thyroglobulin on the basis of the second conjugate content;

(f) calculating a ratio of the amount of the first type of thyroglobulin measured in (d) to the amount of total thyroglobulin; or the amount of second type of thyroglobulin measured in (e) to the amount of total thyroglobulin; and

(g) determining whether a thyroid tumor is malignant or benign by comparing the calculated ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) and (ii),

(i) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid, or

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, and

the sample is determined to be benign in the following case (iii):

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the malignant thyroid.

Claim 75 (Previously Presented): A method for determining between a malignant thyroid tumor and a benign thyroid tumor comprising:

(a) dividing a fluid originating from a living body into a first portion and a second portion;

(b)(i) adding to the first portion a specific lectin capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin,

to form a conjugate of the first type of thyroglobulin with the specific lectin; then

(ii) adding to the first portion an antibody-2 capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin is already bound, to form a conjugate of the second type of thyroglobulin with the antibody-2; and

(iii) measuring the amount of the second type of thyroglobulin on the basis of the measurement of the second type of thyroglobulin with antibody-2 conjugate formed in step (b)(ii); and

(c)(i) measuring an amount of the total thyroglobulin of the second portion; and

(ii) determining an amount of the first type of thyroglobulin from the difference between an amount of the total thyroglobulin and the amount of the second type of thyroglobulin obtained in step (b)(iii);

(d) calculating a ratio of the amount of the first type of thyroglobulin measured in (c)(ii) to the amount of total thyroglobulin; or the amount of second type of thyroglobulin measured in (b)(iii) to the amount of total thyroglobulin; and

(e)determining whether a thyroid tumor is malignant or benign by comparing the calculated

ratio with corresponding predetermined ratios from a reference fluid sample originating from a living body having a normal thyroid and a reference fluid sample originating from a living body having a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) and (ii),

(i) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid, or

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, and

the sample is determined to be benign in the following case (iii):

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the malignant thyroid.

Claim 76 (Canceled).

Claim 77 (Previously Presented): The method according to any one of claims 59 and 68-75, wherein the sugar chain with the specific structure is one found in thyroglobulin which is produced by a carcinoma cell.

Claim 78 (Previously Presented): A method for determining between a malignant thyroid tumor and a benign thyroid tumor comprising the steps of:

(a) adding to a fluid sample originating from a living body;

(i) a specific lectin capable of binding to a specific structure of a sugar chain of a first type of thyroglobulin but not capable of binding to a sugar chain of a second type of thyroglobulin ,

(ii) a first anti-thyroglobulin antibody, capable of binding to both types of thyroglobulin, and

(iii) a second anti-thyroglobulin antibody, capable of binding to the two types of thyroglobulin, but not capable of binding to the thyroglobulin to which the specific lectin is already bound,

to form a first conjugate which is a conjugate of the first anti-thyroglobulin antibody with the first type of thyroglobulin and with the specific lectin, and a second conjugate which is a conjugate of the first anti-thyroglobulin antibody with the second type of thyroglobulin and the second anti-thyroglobulin antibody;

(b) measuring an amount of the first type of thyroglobulin; and

(c) measuring an amount of the second type of thyroglobulin;

(d) calculating a ratio of the amount of the first type of thyroglobulin measured in (b) to the amount of total thyroglobulin; or the amount of second type of thyroglobulin measured in (c) to the amount of total thyroglobulin; and

(d) determining whether a thyroid tumor is malignant or benign by comparing the

calculated ratio with a corresponding predetermined ratio from a reference fluid sample originating from a living body having a normal thyroid or a benign thyroid;

wherein the sample is determined to be malignant in any of the following cases (i) and (ii),

(i) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly higher than that of the reference fluid sample of the benign thyroid, or

(ii) when the calculated ratio is significantly lower than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the benign thyroid, and

the sample is determined to be benign in the following case (iii):

(iii) when the calculated ratio is significantly higher than that of the reference fluid sample of the normal thyroid and is significantly lower than that of the reference fluid sample of the malignant thyroid.

IX. EVIDENCE APPENDIX

There is no additional evidence being submitted.

X. RELATED PROCEEDINGS APPENDIX

The appellants know of no other related proceedings.